DESCRIPTION

THE MANUFACTURING OF IRON DEFICIENT RESISTANT GRASSES

Technical Field

The present invention relates to a manufacturing method for gramineae that have an iron deficiency resistance, gramineae obtained through the method, the method of growing said gramineae and the crop obtained through the method.

More specifically, the present invention relates to the creation of gramineae having an iron deficiency resistance by introducing genes, in which the gene codes an enzyme along the mugineic acid synthesizing route for gramineae, and more preferably, where said enzyme is nicotianamine amino transferase.

Background Art

90% of the soil on the earth is inadequate soil that has some kind of problem. The inadequate soil, in general, lacks the elements essential to the growth of plants qualitatively and quantitatively, and therefore, the growth of plants is hindered or growth disorders occur due to the soil containing a large amount of heavy metals. Representative of inadequate soil is dryland salt accumulated soil. Of this type, there are ones in which NaCl and Na_2CO_3 are accumulated or $CaCO_3$ or $CaSO_4$ are accumulated in the topsoil due to artificial over-irrigation or dry weather over a long period of time. The halomorphic soil causes a salt density disorder, and the calcareous soil causes an iron deficiency disorder.

Approximately 30% of the cultivated soil on the earth is said to be a potentially iron deficient area. . (~~Wallece~~ Wallence et al. "Iron Chlorosis in Horticultural Plants," 75 American Society for Horticultural Science, 819-839 (1960)). The calcareous soil in semiarid areas has calcareous components eluted from the core material due to a capillary effect and it

is accumulated on the surface of the ground. In this soil, the pH is increased and becomes alkaline, and therefore, the iron in the soil exists in the form of Fe $(OH)_3$ and has extremely low solubility.

The plants grown in these soils have iron deficient chlorosis due to little soluble iron, and their growth is hindered or they die.

The iron obtaining system of higher plants is classified into two types, Strategy I and Strategy II. Strategy I is an iron obtaining system for higher plants excluding gramineae. It is a system in which the insoluble trivalent iron in the soil is reduced by the trivalent iron reduction enzyme that is present on the surface of the cell on the root, and then is it absorbed by the divalent iron transporter. Of those plants that have this system, there are ones that have a system to emit protons in the rhizosphere to increase the activity of the trivalent iron reduction enzyme by lowering the pH in the rhizosphere, and ones that have a system to emit phenol compounds in the rhizosphere and supply the Fe (III) to the trivalent reduction enzyme that is present on the surface of the cell by an Fe (III)-phenol compound chelate. Recent studies have isolated the divalent iron transporter IRT1 (Eide et al., "A novel iron-regulated metal transporter from plants identified by functional expression in yeast," 93 Proc. Natl. Acad. Sci. 5624-5628 (May 1996)) that distinctively emerges on the root of the arabidopsis thaliana, and the gene for the trivalent reduction enzyme of the arabidopsis thaliana. (Robinson et al., "The froh gene family from Arabidopsis thaliana: Putative iron-chelate reductases", 196 Plant and Soil 245-248, Kluwer Academic Publishers (1997)).

Strategy II is an iron obtaining system that is only observed in gramineae, which is one of the monocotyledons. The gramineae emits mugineic acids that have trivalent iron chelate activity under iron deficient conditions, and absorbs iron from the root as an "Fe (III)-mugineic acid" complex. (Takagi et al., "Physiological aspect of magineic acid, a possible phytosiderophore of graminaceous plants," 7(1-5) Journal of Plant Nutrition 469-477 (1984)).

There are 7 mugineic acids (MAs) that are known: mugineic acid (MA), 2'-deoxymugineic acid (DMA), 3-hydroxymugineic acid (HMA), 3-epihydroxymugineic acid (epiHMA), avenin acid (AVA), distichon acid and epihydroxydeoxymugineic acid (epiHDMA). All of the mugineic acids (MAs) are, as shown in FIG. 1, synthesized with ~~methyonin~~ methionine as a precursor. (Shojima et al., "Biosynthesis of Phytosiderophores", 93 Plant Physiol. 1497-1503 (1990) and Ma et al., "Biosynthesis of Phytosiderophores in several Triticeae species with different genomes," Vol. 50, No. 334, pp. 723-726, Journal of Experimental Botany, (1999)).

The excretion of mugineic acid has a circadian rhythm (Takagi et al. *supra*) and its excretion reaches a maximum after ~~sunset~~sunrise, and there is no excretion during the night. In addition, it has been observed that the granule expands before the excretion in iron deficient barley root and wilts after the excretion (Nishizawa et al., "The particular vesicle appearing in barley root cells and its relation to mugineic acid secretion," 10(9-16) Journal of Plant Nutrition 1013-1020 (1987)). Therefore, it is believed that the mugineic acid is synthesized in this granule. These fact indicates that the responding of the gramineae to the iron deficiency is formed by not only the synthesis of the mugineic acid but also is formed by a complicated system such as the transmission of an iron deficiency signal and changes in the root form.

It has been reported that a gene for the nicotianamine synthesizing enzyme, which is an enzyme related to the mugineic acid synthesizing route, has been isolated and it is induced by an iron deficiency. (Higuchi et al., "Cloning of Nicotianamine Synthase Gene, Novel Genes Involved in the Biosynthesis of Phytosiderophore," 119 Plant Physiology 471-479 (02/1999)). In addition, the gene for nicotianamine amino transferase~~genes~~ (NAAT) has been isolated and it is induced by an iron deficiency. (Takahashi et al., "Purification, characterization and DNA sequencing of nicotianamine aminotransferase (NAAT-III) expressed in Fe-deficient barley roots," Plant nutrition, 279-280, Kluwer Academic

Publishers (1997))

Moreover, through differential screening using mRNA extracted from an iron deficient barley root and a control barley root, genes Ids1, Ids2, and Ids3 which were specifically induced under iron deficient conditions have been isolated. The *Ids*1 is a gene that codes for metallothionain protein. (Okumura et al., "An iron deficiency-specific cDNA from barley roots having two homologous cysteine-rich MT domains," 17 Plant Molecular Biology 531-533, Kluwer Academic Publishers (1991)). *Ids*2 is a gene in which the sequence of amino acids that is assumed from its genetic sequence is homologous to the hydroxide enzyme. (Okumura et al., "A dioxygenase gene (Ids2) expressed under iron deficiency conditions in the roots of Hordeum vulgare", Plant Molecular Biology 25; 705-719, Kluwer Academic Publishers, (1994)) *Ids*3 is also a gene in which the sequence of amino acids that is assumed from its genetic sequence is homologous to the hydroxide enzyme. (Nakanishi et al., "Expression of a Gene Specific for Iron Deficiency (Ids3) in the Roots of Hordeum Vulgare", 34(3) Plant Cell Physiol 401-410, JSPP (1993)) There are two hydroxide reactions along the epihydroxymugineic acid synthesizing route, and this gene is believed to code the enzyme that catalyzes this reaction.

In addition, the examples of proteins that are induced by an iron deficiency of the barley root are, the IDS3 protein, adenin-ribose-phosphate transferases (Itai et al., "Induced activity of adenine phosphoribosyltransferase (APRT) in iron-deficient barley roots: a possible role for phytosiderophore production". Vol. 51, No. 348, pp. 1179-1188, Journal of Experimental Botany (July 2000)), formicacid dehydrogenate enzyme (Suzuki et al., "Formate Dehydrogenase, an Enzyme of Anaerobic Metabolism, is induced by Iron Deficiency in Barley Roots," 116 Plant Physiol 725-732 (1998)), and 36kDa protein (Tomohiro Irifune, "Partial amino acid sequences of a specific protein in iron-deficient barley roots", (1991)). Gramineae biosynthesizes mugineic acid under iron deficient conditions. This

time, it is believed that the ~~methyonin~~ methionine contained in the root is reduced so that methionine ~~methyonin~~ is synthesized during a methionine ~~methyonin~~ cycle and at the same time, in order to convert the generated ~~adnin~~ adenine into AMP, adenine-ribose-phosphate transferases are induced. (Itai et al., *supra*)

The formic acid dehydrogenate enzyme decomposes formic acid generated during the methionine ~~methyonin~~ cycle. It was reported that the root of a gramineae with an iron deficiency has a deformation of the mitochondrion and a reduction of the energy charge of the electron transmission system (Mori et al., "Why are young rice plants highly susceptible to iron deficiency", Iron nutrition and interactions in plants, 175-188, Kluwer Academic Publishers (1991)). It is believed that the formic acid dehydrogenate enzyme is induced by the anaerobic condition generated by the iron deficiency, and that NADH is supplied as an energy source.

Along with the increase in population, an increase in food production is a significant issue as a condition for human existence in the future. Gramineae has been one of the most important foods since ancient times, however, in reality, the growth of gramineae is difficult in areas with iron deficiencies. If it is possible to grow graminae in an area with an iron deficiency, an increase in food production would be possible, thus it has been attracting people's attention as one of the solutions to increase food production.

Disclosure of the Invention

The present invention has as an objective to provide gramineae with iron deficiency resistance, which can be grown in areas with iron deficiencies.

More specifically, the present invention has as an objective to provide graminae with an iron deficiency resistance that vigorously grows even in a calcareous alkaline soil by introducing the gene of an enzyme in the biosynthesis of the mugineic acid of gramineae to

the gramineae.

The present invention relates to a manufacturing method for graminenae with improved iron absorbency by introducing a gene that codes an enzyme on the mugineic acid biosynthesis route to the gramineae. and more specifically, a gramineae with improved iron absorbency through the introduction of the gene *naat*, wherein said enzyme is nicotianamine amino transferase (NAAT).

In addition, the present invention relates to the gramineae which can be manufactured by the method indicated above. Specifically, the present inventrion relates to the gramineae with improved iron absorbency by introducing a gene that codes an enzyme on the mugineic acid biosynthesis route to the gramineae, more specifically, the gramineae with improved iron absorbency through the introduction of the gene naat that codes the enzyme, wherein said enzyme is nicotianamine amino transferase (NAAT).

Furthermore, the present invention pertains to a growing method of said gramineae with improved iron absorbency and crops obtained through said growth.

Brief Description of Drawings

The patent or application file contains at least one photograph executed in color. Copies of this patent or patent application publication with color photograph(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 shows the mugineic acids' biosynthesis route for a barley root with an iron deficiency and its rhizospheric environment.

FIG. 2 shows the genetic sequence of the binary vector pIG121Hm for a gramineae transformation in which the cDNA of naat-A is inserted.

FIG. 3 is a photo in place of a drawing that shows the results when detection of the introduced gene is carried out by the Southern Hybridization method. WT in FIG. 3 shows a

case of an autochthon gramineae, and the control shows a control gramineae in which only the vector was introduced. 1-5, 1-6, 1-7, 8-1 and 15-2 show transformants having a 35S promoter.

FIG. 4 shows the result of measurement of NAAT activity in a root cultivated in a hydroponic solution in the presence of iron (+Fe) and an iron deficiency (-Fe). In FIG. 4, the whited out portion shows the case for +Fe and the shaded portion shows the case of -Fe. WT shows an autochthon type and 1-5, 1-6 and 1-7 show transformants.

FIG. 5 is a photo in place of a drawing that shows the growing state of each gramineae which is 8 weeks after a transplanting to alkaline soil. The control in FIG. 5 shows the control gramineae in which only the vector is transplanted and the gramineae on the right is the one that is transformed.

FIG. 6 is a graph that shows a transition of the height of each gramineae after being transplanted to alkaline soil. A black dot shows the transformer 15-2, a black square shows the transformer 8-1, and a white dot shows the control gramineae in which only the vector was transplanted.

FIG. 7 shows a ~~limited enzyme~~restriction map of a phage DNA including an isolated genome *naat*. In FIG. 7, E indicates *Eco*RI, H indicates *Hin*dIII, B indicates *Bam*HI, and N indicates *Not*I. The *Not*I site on both sides is the *Not*I located at the arm of λFIXII.

FIG. 8 shows the genetic sequence of the binary vector pBIGRZ1 for a transformed gramineae in which a fragment of the NAAT genome is inserted. In FIG. 8, NPTII is a kanamycin resistant gene, HPT is a hygromycin resistant gene, GUS is a β glucuronidase gene with intron, LacZ is a β galactosidase gene, 35P is a 35S promoter, NP is an NOS promoter, NT is an NOS terminator, MCS is a multi-cloning site, and Riori is an Ri plasmid replication starting point.

FIGS. 9A through 9D show the base sequence (SEQ. ID No. 3) of the obtained

genome *naat*.

FIGS. 10A through 10G show the base sequence of *naat* (SEQ. ID No. 3) and 5' upstream of *naat-A* and ~~*naat*~~*naat-B*, the exon, the intron and 3' downstream, which were determined by comparing with the cDNA. In FIGS. 10A through 10G, the uppercase letters show the exon portion that is a transcription on the cDNA (SEQ. ID No. 1 and 2) and the lowercase letters show the rest.

FIG. 11 is a schematic view of the obtained genome fragment. In FIG. 11, E is *Eco*RI, H is *Hin*dIII and B is *Bam*HI.

FIG. 12 shows the location and the size of the intron in the cDNA of *naat-A* and *naat-B*~~*naat*~~.

FIG. 13 shows an amino acid expressed in a single letter code of an amino acid sequence of NAAT-A (SEQ. ID No. 1) estimated from the cDNA.

FIG. 14 shows an amino acid expressed in a single letter code of an amino acid sequence of NAAT-B (SEQ. ID No. 2) estimated from the cDNA.

FIGS. 15A and 15B are photographs in place of drawings that shows the growing state of each gramineae in which a genome naat was introduced, ten (10) weeks after being transplanting to an alkaline soil. The control in FIGS. 15A and 15B shows the control gramineae in which only the vector is transplanted and the gramineae on the right is the one that is transformed with a genome *naat*.

FIG. 16 is a graph that shows the transition of the height of each gramineae in which a genome *naat* was introduced after being transplanted to alkaline soil. In FIG. 16, the gramineae on the left is the one transformed with genome *naat* and the one on the right is the control gramineae in which only the vector was transplanted.

Best Mode for Carrying Out the Invention

Said Strategy II, which is an iron obtaining system observed only in gramineae, from among the monocotyledon, utilizes a method of biosynthesizing and emitting mugineic acids to obtain iron. Therefore, the enhancement method of enzymes along the biosynthesis route of mugineic acids (see FIG. 1) was investigated.

The present inventors first paid attention to nicotianamine amino transferase~~gene~~ (NAAT) as the enzyme on the mugineic acid synthesizing route, and then attempted to introduce the gene naat. Miraculously, it was found that gramineae with the gene introduced were able to vigorously grow even in a calcareous alkaline soil.

The cDNA of the naat-A, the nicotianamine amino transferase~~genes~~ (NAAT), was integrated into pIG121Hm using the *Xba*I and *Sac*I portion and the binary-vector shown in FIG. 2 was created. The obtained vector was used for transformation by introduction into an agro-bacterium.

The transformation of the gramineae was carried out in accordance with the method by Hiei et al., "Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA", 6(2) The Plant Journal 271-283, (1994), and "*Tsukinohikari*" was used as the material. The callus induced from the blastoderm was immersed and infected in said transformed agro-bacterium suspension solution, and a regenerator (T1 plant) was obtained. Then, finally, the 34 strain transformed gramineae was obtained from seed.

The introduced gene was detected by the Southern Hybridization method. The results are shown in FIG. 3. In FIG. 3, WT shows a case of an autochthon gramineae and the control shows a case of a gramineae in which only the vector was introduced. 1-5, 1-6, 1-7, 8-1 and 15-2 show the transformer, which has a 35S promoter. As shown in FIG. 3, all the transformers have an over-generation of *naat-A*. In addition, it was found that among those

35S transformed gramineae, 8-1 and 15-2 had at least 5 copies and 2 copies of naat introduced, respectively.

By introducing the genetic naat, it is assumed that compared to the autochthon and one in which is it only introduced on the vector, there is an over-emission of nicotianamine amino transferase (NAAT) and as a result, the mugineic acid synthesizing route is activated, and consequently, the mugineic acids which are required for iron intake was massively produced.

Therefore, first, the NAAT activity of these species was investigated. Young plants (T2), 3 weeks after sprouting, were cultivated for 2 weeks in a hydroponic solution with the presence of iron (+Fe) and an iron deficiency (-Fe). The results of measurement of NAAT activity is shown in FIG. 4. In FIG. 4, the whited out portion shows the case of +Fe and the shaded portion shows the case of -Fe. WT shows the autochthon type and 1-5, 1-6 and 1-7 show the transformers.

As a result, for both +Fe and -Fe, the transformed ones had higher relative activity than the non-transformed autochthon one (WT), and in addition, it was found that the relative activity further increased with the -Fe condition. This shows that not only the introduction of a gene allows the high activity of NAAT, but also, the transformer is significantly promoted with NAAT activity in the presence of an iron deficient state or conditions with an insoluble iron. In other words, it is assumed that it has become a species with a strong resistance to an iron deficient condition or the condition of insoluble iron.

From the above, it was found that the introduction of a gene *naat* promotes NAAT activity. Nonetheless, whether these transformers can be grown in actual iron deficient soil was investigated. When 35S-*naat-A* transformed gramineae was transplanted to an alkaline soil in the condition of insoluble iron, its leaves turned yellow up to 2 weeks after the transplant, however, after 4 to 5 weeks, the new leaves became a dark green and started to

recover. FIG. 5 is a photo showing the growth state 8 weeks after it was transplanted to alkaline soil. In FIG. 5, the control shows the control gramineae in which only the vector was transplanted and the gramineae on the right shows the transformed one. It is found in comparison to the control one, that the transformed one has significantly superior growth. In addition the transition of the plant height after it was transplanted to the alkaline soil is shown in FIG. 6. In the graph in FIG. 6, the Y axis shows the height of the plant (cm) and the X axis shows the number of days after it was transplanted to the alkaline soil. Black dots show transformer 15-2, black squares show transformer 8-1 and white dots show the control gramineae in which only the vector was transplanted.

As described above, 35S transformed gramineae 8-1 has at least 5 copies of *naat* genes, and 35S transformed gramineae 15-2 has at least 2 copies of *naat* genes introduced. From the height of the plant in FIG. 6, the number of copies of the gene is not related and it shows that as long as the gene was introduced, the gramineae has gained an iron deficiency resistance.

As described above, the introduction of the gene increases the activity of the enzyme along the mugineic acid synthesizing route of the gramineae, and furthermore, it was found that by doing so, it added an iron deficiency resistance.

For the enzyme along the mugineic acid synthesizing route of the present invention, it is acceptable as long as it is an enzyme along the mugineic acid biosynthesizing route shown in FIG. 1, and the introduction of the gene that codes said enzyme increases its activity. As described above, among the candidates, nicotianamine amino transferase (NAAT) and ~~nicotinamine~~ nicotianamine synthesizing enzyme are desirable. The gene that codes the enzyme along the mugineic acid synthesizing route of the gramineae of the present invention can be either cDNA or one derived from the genome. As described in the later section, the use of a genome is a preferable example of the present invention.

Therefore, for the gramineae of the present invention, it is acceptable as long as it is a plant that can absorb iron through the Strategy II system, and it is not limited to gramineae in the academic sense. The preferable examples of gramineae of the present invention are, rice plants, corn, sorghum, wheat, barley, and oats. These gramineae can have the method of the present invention applied regardless of its species, and the gramineae with the target gene introduced can be manufactured.

The promoter of the present invention is not limited as long as it can generate the target enzyme. 35S promoter, and more specifically, CaMV35S promoter can be used.

The vector of the invention is not specifically limited as long as it can preferably be used during the transformation. The transformation method of the present invention is not limited to said method that uses the agro-bacterium method and a variety of transforming methods using particle guns, etc., can be employed. The cells of the gramineae formed are not limited to the cell from said callus and a variety of cells can be used, however, normally it is preferable to use ones derived from the callus.

The iron deficiency related to the present invention can be a state where iron is deficient, but preferably it is a state where the form of the iron is one that the plant has problems absorbing, and depending on the type of plant, it can be determined whether it is deficient of iron or not. Therefore, the definition of the iron deficiency resistance in the present invention is that the resistance is to the difficult conditions where the plants of the subject have difficulty absorbing the iron in the soil.

Next the present inventor attempted an introduction of a genome *naat* of barley instead of the cDNA of the *naat*.

For the genome *naat*, the library (manufactured by Srtratagen Corp.) created using the genome DNA extracted from barley (*Horudeum vulgare L. var. Igri*) was used. This library was partially cut by ~~limiting the~~ restriction enzyme Sau3AI and was introduced to the *Xho*I

site of the λFIXII vector. For the probe, the entire cDNA of the *naat-A*, which was isolated in advance was used. An *Escherichia coli* XL1-Blue MRA (*E. coli XL1-Blue MRA (P2)*) was used as the host.

As the result of screening, five (5) phages were obtained. From these, each of the phage DNA was isolated and a ~~limiting enzyme~~restriction map was created. It was found that the same fragments were increased for all the cases. Namely, it was found that the obtained five phages were derived from the same portion of the genome. It was assumed that it contains the *naat* used in the probe. Therefore, the base sequence determination for the one of them shown in FIG. 7 was carried out.

The phage DNA shown in FIG. 7 is inserted in the *Not*I site of the plasmid vector-pBIGRZ1 in which 10 kb or more fragments can be inserted and a transformation of the gramineae using *agro-bacterium* can be carried out. (See FIG. 8.)

In addition, up to 11.0 ~~kg~~ kb of the fragments shown in FIG. 7 were divide into 4 parts, that is A to D, and they were introduced to the *Eco*RI site (B, C) of the plasmid vector-pBluescript SK~~(+)~~(-) or the *Not*I and the *Eco*RI site (A, D).

For fragments A to D, the base sequence was determined from both sides of the fragment using a primer based on the sequence on the plasmid (M13 forward primer, M13 reverse primer). To determine the DNA base sequence, the DNA sequencer DSQ-2000L of Shimadzu, Ltd. was used.

The details of the sequence determination are shown in the examples.

Sequence Identification No. 3 in the sequence listing shows the determined 10,966 bp base sequence. In addition, the entire sequence is shown in FIGS. 9A through 9D (without base number).

From the obtained base sequence, this 10,966 bp gene is found to be a fragment of the barley genome that codes the *naat-A* and ~~*naat*~~*naat-B* that have been obtained. It was in the

order of ~~*naat*~~ *naat-B* and *naat-A*.

5' upstream *of naat-A* and ~~*naat*~~*naat-B*, the exon, the intron and 3' downstream were determined by comparing with the 10th cDNA as shown. In FIG. 10, the upper case letters show the exon portion transcribed to the cDNA, and the lower case letters show the rest. The base number of the exon portions are as follows.

FIG. 11 shows the schematic view thereof. The exon portion is shown in the shaded portion. Both genes comprise 6 intron and 7 exon. In addition, the insertion location of the intron was homologous for each of the genes. FIG. 12 shows the location in the cDNA and what size of intron was inserted.

The amino acid sequence of *naat-A* and ~~*naat*~~ *naat-B* estimated from the cDNA, is shown in FIGs. 13 and 14, respectively.

The transformation method of gramineae, in which an obtained barley genome *naat* is introduced, was carried out in accordance with the transformation method of said 35S transformed gramineae.

Next, the inspection of the iron deficiency resistance of the gramineae introduced with the obtained genome *naat* was carried out. Of the obtained regenerators (T 1), 39 individuals and 15 individual controls, in which only the vector was introduced, were used, and the inspection was carried out in a similar manner to the 35S transformed plant. From the 5th week after the transplantation, the height of the plants was measured every week or every other week. Twice every 4 to 5 weeks, they were transplanted to a pot with increased soil.

The leaves of the transformed gramineae with the genome *naat* turned yellow by the second week after they were transplanted to the alkaline soil, however, during the 4th to 5th week, new leaves started to become dark green and recover, and then they started to show vigorous growth. Compared to this, the control group in which only the vector was introduced continued to have yellow leaves for a long period of time, and from around the 8th

week, new leaves started to turn green.

FIG. 15 is a photo that shows the growth state of 10th week after it was transplanted to the alkaline soil. The control in FIG. 15 shows the control gramineae in which only the vector was transplanted. The gramineae on the right is the one transformed with the genome *naat*.

FIG. 16 shows the transition of the plant height after it was transplanted to the alkaline soil. In the graph of FIG. 16, the Y axis shows the plant height (cm), and the X axis shows the number of days after it was tranplanted to the alkaline soil. In FIG. 16, the gramineae on the left shows the gramineae transformed with a genome *naat* and the one on the right shows a control gramineae in which only the vector was transplanted.

From the above, it was found that the introduction of the genome *naat* allows the gramineae to have additional iron deficiency resistance.

Examples

The present invention is described in detail using examples as follows, however, the present invention is not limited to these.

Example 1: Transforming Method of Gramineae in Which There is an Over-developing *Naat* with CaMV35S Promoter

The binary vector shown in FIG. 2 was created by integrating the cDNA of the genetic *naat* to pIG121Hm, using the *Xba*I and *Sac*I portions. These were introduced to agro-bacterium and used for the transformation.

The transformation of gramineae was carried out in accordance with the method of Hiei et al., "Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA", 6(2) The Plant Journal 271-283, (1994)

and "*Tsukinohikari*" was used as the material. First, hulled seeds were sterilized and seeds on a callus-inducing medium (pH 5.8) comprising N6 inorganic salt, 30 g/L of N6 vitamin, 2 mg/L of sucrose, 2, 4-D, and 2 g/L gelrite were cultured for 3 weeks at 25 °C under the conditions of 60 μmol/~~m2s~~ $m^{2-}s$ with a 16-hour photo period/8 hour dark period, and the callus was induced from the blastoderm.

After it was transplanted to the new medium and cultured for 3 days at 25 °C in a bright place, it was immersed in the agro-bacterium suspension with an agro-bacterium suspension medium (pH5.8) (20 g/L of AA inorganic salt, amino acid, B5 vitamin, 2 mg/L of sucrose, 0.2 mg/L of 2, 4-D, 10 mg/L of kinetin and acetosyringone). Then after it was dried with a paper towl, it was infected for 3 days at 28 °C in a dark place in the co-existing culturing medium (pH5.2) (30 g /L of N6 inorganic salt and N6 vitamin, 10 g/L of sucrose, 2 mg/L of glucose, 10 mg/L of 2, 4-D, and 2 g/L of acetosyringone gelrite).

Then, the agro-bacterium was removed by rinsing the callus with a sterilized washing solution with 500 mg/L of Claforan, and it was placed on a selected medium containing 50 mg/L of hygromycin (pH5.8) (30 g/L of N6 inorganic salt and N6 vitamin, 2 mg/L of sucrose, 2 g/L of 2, 4-D, 500 mg/L of gelrite, 50 mg/L of Claforan and hygromycin) and cultured for 3 weeks at 25 °C in a bright place.

After it was cultured, it was transferred to a redifferentiation medium (pH5.8) (30 g/L of MS inorganic salt and MS vitamin, 30 g/L of sucrose, 2g/L of sorbitol, 1 mg/L of casamino acids, 2 mg/L of NAA, 500 mg/L of BAP, 50 mg/L of Claforan, 4 g/L of hygromycin and gelrite) and the regenerator (T1 plant) that was redifferentiated in 3 to 5 weeks was transferred to an inspection medium. The inspection medium (pH5.8) comprises 30 g/L of MS inorganic salt and MS vitamin, 50 mg/L of sucrose, 8 g/L of hygromycin, and agar.

The plants that grew to fill the petridish were established for 4 to 5 days, transferred to a soil mixed with a synthetic culture soil (BONSOL 1, Sumitomo Chemical Co., Ltd.) and

Vermiculite in a 1:1 ratio and seeds were obtained. Consequently, a 34 strain transformed gramineae was obtained.

Example 2: Detection of the Introduced Gene Using the Southern Hybridization Method

The leaves of the T1 plant obtained in Example 1 were ground and the genome was extracted by a modification of the C-TAB method. The extracted genome was treated with *Hind* III and separated by electrophoresis using a 0.8% agarose gel. These were blotted on a nylon membrane. Using the primer created with the internal sequence of the *naat* on a probe, and one labeled with ~~32 P~~ ^{32}p by PCR, hybridization was carried out. Then the band was detected using BAS 2000 (Fuji Photo Film Co., Ltd.).

The results of this Southern Hybridization are shown in FIG. 3. In FIG. 3, WT shows the autochthon gramineae, and the control shows a control gramineae in which only the vector was introduced. 1-5, 1-6, 1-7, 8-1 and 15-2 are cases in which the gramineae has an over-emergence of *naat-A* with the 35S promoter.

From the results shown in FIG. 3, it was found that of the 35S transformed gramineae, at least 5 copies and 2 copies of *naat* were introduced to the 8-1 and 15-2, respectively.

Example 3: Inspection of Iron Deficiency Resistance Using Alkaline Soil

As a sample soil, a fossil shell soil comprised of the following composition was used.

The content of each element is shown as % dry soil after analysis by several methods, dry weight method after ashing, flame photometry,atomic absorption, spectrophotometry and etc. Some elements were shown as chemical compounds. Therefore, summing up all data covers more than 100% because some elements were doubly counted.

Water content	0.48%
Total phosphate	0.12%
Total potassium	0.12%
Total silicate	22.79%
Total lime	37.82%
Total magnesia	0.91%
Total manganese	0.018%
Total boron	0.003%
Alkaline	38.80%
Hydrochloride insoluble substance	28.88%
Iron oxide	0.99%
Aluminum oxide	5.59%
Zinc	0.002%

The soluble iron content of this soil was ~~22~~ 2.2 ppm, the pH was 8.78 and the electric resistance was 0.03 mΩ.

The inspection of the 35S transformed plant was carried out such that, first, the obtained seeds from the regenerator (T1) of the transformed plant were sown on an MS solid medium containing 50 mg/L and selected, and after they were established, young plants (T2) that grew to 20 to 25 cm were used.

The inspection of the resistance was carried out for 16 of the 34 strains having 27 species. The inspection method is as follows: first, a paper towel and a filter were cut in a circle and placed on the bottom of a plastic black pot (0.5 L) and filled with alkaline soil. Plants were transferred to the pot and then from the bottom of the pot placed in a hydroponic solution (Kasugai Solution: 7×10^{-4}M K_2PO_4, 1×10^{-4}M KC1, 1×10^{-4}M KH_2PO_4, 2×10^{-3}M $Ca(NO_3)_2$, 5×10^{-4}M $MgSO_4$, 1×10^{-5}M H_3BO_3, 5×10^{-7}M $MnSO_4$, 5×10^{-7}M $ZnSO_4$, 2×10^{-4}M $CuSO_4$, 1×10^{-8}M $(NH_4)_3MoO_{24}$, 1.5×10^{-4}M Fe-EDTA) at 2 to 3 cm from the bottom of the pot, and grown in a greenhouse at a temperature of 30 °C during the day and 25 °C at

night. The alkaline soil was increased to 1L after 3 to 4 weeks and after 8 to 9 weeks, increased to 2L and transferred. From the second week after the transplantation, the plant height was measured every week or every other week.

The measurement of the NAAT relative activity of the transformed gramineae (35S-naat gramineae) grown in the hydroponic solution of +Fe (iron presence) or -Fe (iron deficiency) was carried out as follows. Young plants (T2), 3 weeks after the sprouting, were grown with an +Fe and -Fe hydroponic solution for 2 weeks, and the NAAT activity at the root of each plant was measured. The results are shown in FIG. 4. In FIG. 4, the whited out portion shows the case for +Fe, and shaded portion shows the case for -Fe. WT shows the autochthon case and 1-5, 1-6 and 1-7 show the transformed cases.

In both the +Fe and –Fe case, the transformed one had a higher relative activity than the non-transformed autochthon (WT) and the relative activity was even higher in the case of -Fe. (See FIG. 4)

Example 4: Inspection of iron Deficiency Resistance Using Alkaline Soil

35S-*naat-A* transformed gramineae were transferred to an alkaline soil and their growth was observed.

The leaves of the 35S-*naat-A* transformed gramineae turned yellow until the second week after the transplant, however, on the 4th to 5th week, the new leaves turned to a deep green and recovered. Thus it was found that the introduction of *naat* allows the gramineae to have an iron deficiency resistance.

FIG. 5 is a photo showing the growth state at 8 weeks after the transplant to the soil. In FIG. 5, the control shows the control gramineae in which only the vector was transplanted. The gramineae on the right is transformed.

FIG. 6 shows the transition of the plant height after being transplanted to the alkaline

soil. In the graph of FIG. 6, the Y axis shows the plant height (cm) and the X axis shows the number of days after it was transplanted to the alkaline soil. Black dots show transformer 15-2, black squares show transformer 8-1 and white dots show the control gramineae in which only the vector was transplanted.

~~It~~It was found that by introducing a gene naat, an iron deficiency resistance could be added to the gramineae. (See FIGs. 5 and 6.)

Example 5: Isolation of *Naat-A* and *B* Genomic Clone

The screening procedure was carried out in accordance with Itaru Watanabe, "*Cloning and Sequence*", Nosonbunka (1989).

The λ FIXII library purchased from ~~Stratagnen~~ Srtratagene Corp. was used as the library. This is created using genome DNA extracted from barley (*Horudeum vulgare L. var. Igri*). The genome DNA was partially cut by the ~~limiting~~ restriction enzyme Sau3AI, and introduced to the *Xho*I site of the λ FIXII vector. The insertion size of the library was 9 to 23 kb.

(1) *E. coli* (XL1-BLUE MRA (P2)) was cultured overnight in a NZCYM liquid medium, (10 g of NZ amine, 5 g of NaCl, 1 g of casamino acid, 5 g of Bacto-yeast extract, 2 g of MgSO4 7H2O, and approximately 6 mL of 1N NaOH was diluted with 1 L of distilled water (pH 7.5) and sterilized with an autoclave) then centrifuged and then it was suspended in 20 mL of 10mM $MgSO_4$ solution.

(2) 100 mL of this E-coli suspension solution and 100 mL of phage dilution (the amount in which 25,000 plaque are created on the plate for screening (9cm x 13 cm)) were mixed and left for 20 minutes at 37 °C, then it was mixed with 8 mL of 50 °C 0.7% top agar (0.7 g of agarose was added per 100 mL) and sown on the plate for screening (9 cm x 13 cm). The plate was left at 37 °C and then it was cultured until the size of the plaque reached 0.5

mm.

(3) A nylon membrane, HYBOND-N (Amersham Corp.) was cut to the size of the plate and then placed on top of topagarose for 30 seconds. This was placed on a filter dipped with a denaturation solution (0.5 M NaOH, 1.5 M NaCl) with the side that came in contact with the plaque up. A second membrane was placed on the topagarose, and left for one minute. Similarly, it was placed on the filter dipped in the denaturation solution. After it was left for 5 minutes, the second membrane was moved onto a filter dipped with a neutralization solution (0.5M Tris-HCl, pH 8.0, 1.5M NaCl). After it was left for 5 minutes, it was well washed twice with 2xSSPE (0.02M, NaH_2PO_4 pH 7.4, 0.3M NaCl, 2 mM EDTA) and then dried.

(4) In order to use the whole length of the cDNA of the isolated *naat-A* in advance as a probe, those that were at the site of the *Hin*dIII of the plasmid vector pYH23 and the *Not*I sites were cut out and purified. These were labeled with ~~[α-^{32}P]~~ [α-^{32p}] dATP using a RANDOM PRIMER DNA ~~LABELLING~~ LABELING KIT VER. 2 (Takara Shuzo Co., Ltd.)).

~~Prehybridizaqtion~~ Prehybridization was carried out for 1 hour at 65 °C with 30 mL of hybridization buffer (6 x SSPE、 5 x Denhart solution, 0.1% SDS, 100 mg/ mL altered salmon spermary DNA) that was preheated to 65 °C, and the hybridization buffer was replaced (25 mL).

The probe prepared as described above was added to this, and hybridization was carried out for 12 hours at 65 °C. The membrane was cleaned with a cleaner (5x SSPE) heated to 65 °C in advance twice for 10 minutes, and once with a highly stringent cleaner (2 x SSPE, 0.1% SDS) at 65 °C. The membrane was wrapped with Saran Wrap, and photosensitized overnight to an imaging plate (Fuji Photo Film Co., Ltd.) and results were obtained with an imaging analyzer (Fuji Photo Film Co., Ltd.).

The reagent used is such that 20 x SSPE (0.2 M NaH_2PO_4 pH 7.4, 3 M NaCl and 20

mM EDTA), 50 x denhart solution, 5g of Ficoll 400, 5g of polyvinylpyrrolidone (MW 360,000) and 5g of calf serum albumin were dissolved in 500 mL of distilled water and filtered with a 0.45 mm filter.

(5) What emerged on both of the two membranes was determined to be positive and the plaque that corresponded to the location was cut out from the petridish. That which was cut out was placed in an SM solution (50 mM Tris-HCl pH7.5, 0.1M NaCl, 7mM ~~MgSO4~~ $MgSO_4$, and 0.01% gelatin) and stored at 4 °C. Then, using this phage solution, a second and third screening was carried out in a similar manner. In the end 5 phages were obtained.

The phage DNA of each of the five phages obtained as described above was isolated and a ~~limiting enzyme~~ restriction map was created. It was found that the same fragments were increased for all the cases. Namely it was found that all of the obtained 5 phages were derived from the same part of the genome. In addition, it was assumed that the *naat* used for the probe was contained. Therefore the base sequence was determined for one of these. (See FIG. 7.) In FIG. 7, E is *Eco*RI, H is *Hin*dIII, B is *Bam*HI and N is *Not*I. The *Not*I site at both sites is the *Not*I on the arm of λFIXII.

Example 6: Sub-cloning and Determination of the Base Sequence

(1) The phage DNA shown in FIG. 7 is inserted in the NotI site of the plasmid vector-pBIGRZ1 in which the 10 kb or more fragments can be inserted and a transformation of the gramineae using agro-bacterium can be carried out. (See FIG. 8.) 11.0 kbp of fragments, from the first *Not*I site to the *Not*I site located at 11.0 kb of the phage DNA shown in FIG. 7 was cut out and inserted at the *Not*I site of the pBIGRZ1. (See FIG. 8.) In FIG. 8, NPTII is a kanamycin resistant gene, HPT is a hygromycin resistant gene, GUS is a β glucuronidase gene with intron, LacZ is a β galactosidase gene, 35P is a 35S promoter, NP is an NOS promoter, NT is an NOS terminator, MCS is a multi-cloning site, and Riori is an Ri plasmid

replication starting point.

In other words, the base sequence was determined for this 11.0 kb. For the transforming of the rest of the gramineae, this created construct was used.

(2) pBIGRZ1 is stably maintained in the *E. coli* (XL1-BLUE). *E-coli* having this construct were cultured and the plasmid was extracted from here using plasmid adjuster PI-50 α. (Kurashiki Boseki Co., Ltd.)

(3) The fragments up to 11.0 kb that are shown in FIG. 7 are classified into 4 sections of A to D and these were introduced to the *Eco*RI site (B. C) of the plasmid vector of pBluescript SK (-) or the *Not*I and *Eco*RI site (A, D).

(4) The base sequence from both sides of the fragments for the fragments A to D was determined by the primer (M13 forward primer, M13 reverse primer) based on the sequence on the plasmid. The DNA sequencer DSQ-2000L from Shimadzu, Ltd. was used to determine the base sequence.

(5) The primer to read further from the portion of the base sequence was determined for each fragment and created, and the primer to confirm the already-read sequence in reverse was created and the base sequences were determined in series. At the end, the sequence was determined for all the fragments in both directions from 5' and 3'. The sequence of the primers used to determine the base sequence of each fragment is shown as follows.

These primers are labeled with the fluorescein isothiocyanate, FITC, at the 5' edge in order to be used by the DSQ-2000L.

Primers for fragment A

Name: Sequence F-A1F: FITC-5'-gct act agt agt att cct ggt gta g (SEQ. ID No. 4)

Name: Sequence F-A1R: FITC-5'-gga gta cta cta gac tac acc agg a (SEQ. ID No. 5)

Name: Sequence F-A2F: FITC-5'-aca tgc gca tgc atg aat tgc cg (SEQ. ID No. 6)

Name: Sequence F-A2R: FITC-5'-caa ttc atg cat gcg cat gtg cc (SEQ. ID No. 7)

Primers for fragment B

Name: Sequence F-B1F: FITC-5'-ggt caa gta tgc agt atg ttg gaa c (SEQ. ID No. 8)

Name: Sequence F-B1R: FITC-5'-gtt cca aca tac tgc ata ctt gac c (SEQ. ID No. 9)

Name: Sequence F-B2F: FITC-5'-cta gaa gcc tat gga tgt ttc ttt tgg (SEQ. ID No. 10)

Name: Sequence F-B2R: FITC-5'-cca aaa gaa aca tcc ata ggc ttc tag (SEQ. ID No. 11)

Name: Sequence F-B3F: FITC-5'-agt tct tat caa ttt ccg aga tga c (SEQ. ID No. 12)

Name: Sequence F-B3R: FITC-5'-ata gtc atc tcg gaa att gat aag a (SEQ. ID No. 13)

Name: Sequence F-B4F: FITC-5'-agt ggt cac cat gcg gac caa cac c (SEQ. ID No. 14)

Name: Sequence F-B4R: FITC-5'-ggt gtt ggt ccg cat ggt gac cac t (SEQ. ID No. 15)

Primers for fragment C

Name: Sequence F-C1F: FITC-5'-cac cgg cca gtt caa ctg cta cgc (SEQ. ID No. 16)

Name: Sequence F-C1R: FITC-5'-gcg tag cag ttg aac tgg ccg gtg (SEQ. ID No. 17)

Name: Sequence F-C2F: FITC-5'-ttt gga gga gat cca tga cga cat a (SEQ. ID No. 18)

Name: Sequence F-C2R: FITC-5'-tat gtc gtc atg gat ctc ctc caa a (SEQ. ID No. 19)

Name: Sequence F-C3F: FITC-5'-tct tct cat atg cta ctg tgg gga t (SEQ. ID No. 20)

Name: Sequence F-C3R: FITC-5'-tga cat gca aca cag gga cat gag c (SEQ. ID No. 21)

Primers for fragment D

Name: Sequence F-D1F: FITC-5'-cat gct gac gaa gag cga ggt cat a (SEQ. ID No. 22)

Name: Sequence F-D1R: FITC-5'-ccc agg ata tga cct tag tgg ttg g (SEQ. ID No. 23)

(6) For the portion of the sequence that could not be determined completely, the following primers were newly synthesized by using the ABI PRISMTM 310 genetic analyzer that is an automatic DNA sequencer from PerkinElmer Japan, Inc.

Primers for fragment B

Name: Sequence B5F: 5'-gaa tgg caa act ggg tcc gca tta c (SEQ. ID No. 24)

Name: Sequence B5R: 5'-gta atg cgg acc cag ttt gcc att c (SEQ. ID No. 25)

Name: Sequence B6F: 5'-ctg gtt gtt gtg gcc tgg acg aaa c (SEQ. ID No. 26)

Name: Sequence B6R: 5'-gtt tcg tcc agg cca caa caa cca g (SEQ. ID No. 27)

Name: Sequence B7F: 5'-agc aca aac cct acc tat gtt agg c (SEQ. ID No. 28)

Name: Sequence B7R: 5'-gcc taa cat agg tag ggt ttg tgc t (SEQ. ID No. 29)

Primers for fragment C

Name: Sequence C4F: 5'-tgg aat ttc gcc cgg ggc aag gac (SEQ. ID No. 30)

Name: Sequence C4R: 5'-ccc tgt gac aag tgc tct gct acg (SEQ. ID No. 31)

Name: Sequence C5F: 5'-tct ggg atc tca gtg cat cca aca (SEQ. ID No. 32)

Name: Sequence C5R: 5'-gaa gca tat atc agt caa aca taa cc (SEQ. ID No. 33)--.

In addition, in order to determine the junction of the fragments A and B and fragments B and C, the following primers were created. The base sequence was determined for the construct created in (1) using the ParkinElmer Japan, Inc. automatic DNA sequencer ABI PRISMTM 310 genetic analyzer.

Border between fragments A and B

Name: Sequence A-eF: 5'-cac atc ctt tgc ctt gct gaa tat gg (SEQ. ID No. 34)

Name: Sequence B-tR: 5'-cag tag tac taa tta atc acc tta gta gc (SEQ. ID No. 35)

Border between fragments B and C

Name: Sequence B-eF: 5'-cac gat caa cca aag aat gtc ctc c (SEQ. ID No. 36)

Name: Sequence C-tR: 5'-tac ttg tat atg cag ctc cag cac (SEQ. ID No. 37)

(7) The sequence identification number 3 in the sequence listing of the determined 10,966 bp base sequence is shown. The entire sequence is shown in FIGS. 9A through 9D (without base numbers).

From the obtained base sequence, it was found that this 10,966 bp gene is a fragment

of the barley genome that codes the *naat-A* and *naat-B* obtained so far. The order was *naat-B* and *naat-A*.

At the 109th location, 5' upstream, exon, intron, and 3' downstream of naat-A and naat-B were determined by comparing with the cDNA as shown. In the 10th location, uppercase letters represent the exon portion transplanted to cDNA and lowercase letters represent the rest. The base numbers for the exon portion are as follows.

naat-B

First exon 579-1299 (Starting codon 6518)

Second exon 1483-1825

Third exon 1922-2140

Fourth exon 2244-2303

Fifth exon 2761-2916

Sixth exon 3263-3356

Seventh exon 3735-4033 (Ending codon 3868)

naat-A

First exon 6457-6897 (Starting codon 6518)

Second exon 7029-7371

Third exon 7479-7697

Fourth 4 exon 7784-7843

Fifth exon 8285-8440

Sixth exon 8738-8831

Seventh exon 9414-9732 (Ending codon 9547)

This schematic view is shown in FIG. 11. The exon portion is shown as the shaded portion. Both genes are formed with 6 introns and 7 exons. In addition, the location of the insertion of the intron was homological. FIG. 12 shows where in the cDNA, and which size of

intron was inserted. FIGs. 13 and 14 show the amino acid sequence of *naat-A* and *naat-B* estimated from the cDNA.

Example 7: Transforming Method of Gramineae Introduced with a Barley Genome *Naat*

The transformation method of the gramineae introduced with the genome naat of barley obtained in Example 6 described above was carried out in a similar manner to the 35S transformed gramineae except at those points shown in (1) to (3) as follows.

(1) The callus induction was carried out at 28 °C in a dark place, and the callus induction medium comprised 0.3 g/L of N6 inorganic salt and N6 vitamin, 30 g/L of casamino acid, 2 mg/L of sucrose, 2.8 g/L of 2, 4-D, 4 g/L of proline and gelrite (pH5.8).

(2) It was infected with agro-bacterium at 25 °C and a coexisting culture medium comprising 30 g/L of N6 inorganic salt and N6 vitamin, 10 g/L of sucrose, 1 g/L of glucose, 2 mg/L of casamino acid, 20 mg/L of 2, 4-D, 2 g/L of acetosyringone and gelrite (pH5.2). It was carried out with a placement of the filter.

(3) For the selection, a selection medium was used containing 10 mg/L for the first week, 30 μg/L for the next week and 50 mg/L for the last two weeks of hygromycin, and it was cultured at 28 °C in a dark place.

The selection medium comprising 1 g/L of N6 inorganic salt and N6 vitamin, casamino acid, 30 g/L of sucrose, 2 mg/L of 2, 4-D, 250 mg/L of Claforan, 10 to 50 mg/L of hygromycin and 2 g/L of gelrite (pH5.8) was used and a redifferentiation medium comprising 30 g/L of MS inorganic salt and MS vitamin, sucrose, 30 g/L of sorbitol, 2 g/L of casamino acid, 1.1 g/L of MES, 2 mg/L of NAA, 1 mg/L of kinetin, 250 mg/L of Claforan (Hoechst Marion Roussel Ltd. Japan), 50 mg/L of hygromycin, and 4 g/L of gelrite (pH5.8) was used and it was cultured at 28 °C.

Example 8: The Inspection of the Iron Deficiency Resistance of Gramineae with a Genome *Naat* Introduced

The inspection of the iron deficiency resistance of gramineae with a genome *naat* introduced was carried out for 39 individuals out of the obtained regenerators (T1) and 15 controls in which only the vector was introduced in a similar manner to the 35S transformed plant. From the 5th week after the transplant, the height of the plants was measured every week or every other week. They were transferred twice every 4 to 5 weeks to a pot with an increased soil size.

The leaves of the transformed gramineae with a genome *naat* turned yellow by the second week after they were transplanted to an alkaline soil, however, on the 4th to 5th week, the new leaves started to become a dark green and recover, and then started to show vigorous growth. Compared to this, the control group in which only the vector was introduced continued to have yellow leaves for a long period of time, and from approximately the 8th week, new leaves started to turn green. From this fact, it was found that the introduction of *naat* allows the gramineae to have an iron deficiency resistance. (See FIGs. 15 and 16.)

FIG. 15 is a photo that shows the growth state of the 10^{th} week after being transplanted to an alkaline soil.

FIG. 15 shows the control gramineae in which only the vector was transplanted. The gramineae on the right was transformed with a genome *naat*.

The transition of the height of the plants after being transplanted to an alkaline soil is shown in FIG. 16. In FIG. 16, the Y axis shows the height of the plant (cm) and the X axis shows the number of days after it was transplanted to the alkaline soil. In FIG. 16, the one on the left is the gramineae transformed with a genome *naat* and the one on the right is the control gramineae in which only the vector was transplanted.

Through this, it was found that the introduction of a genome *naat* allows an increase

in the iron deficiency resistance for a gramineae.

Industrial Applicability

The present invention provides a new gramineae with iron deficiency resistance and provides a new gramineae that can be grown in a cultivation area with an iron deficiency.

In addition, the present invention provides the new knowledge that a gramineae with improved iron absorbency can be obtained by introducing a gene that codes the enzyme along the mugineic acid biosynthesizing route, to a gramineae.